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Neoveronaea sinensis gen. & sp. nov. from Jiangxi, China

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Abstract—A new hyphomycete genus and species, *Neoveronaea sinensis,* is described from Jiangxi Province, southern China. *Neoveronaea* is characterized by its macronematous conidiophores, and euseptate, obovoid to ellipsoidal, pale brown, smooth conidia. Phylogenetic analyses of partial DNA sequences of internal transcribed spacer (ITS) and nuclear ribosomal large subunit (LSU), using Maximum-Likelihood and Bayesian Inference, reveal the taxonomic placement of *Neoveronaea* within the *Herpotrichiellaceae*, in which it forms a lineage distinct from other genera.

Key words—asexual *Ascomycota*, *Chaetothyriales,* systematics

Introduction

China is located in eastern Asia on the west coast of the Pacific Ocean. It is considered an important reservoir of biodiversity with its unique geography and ecology (Wang 1992). However, its mycobiota, especially saprobic microfungi, are poorly known (Ma & al. 2014, Qiu & al. 2021). During our continuing surveys of asexual fungi occurring on plant debris in forest ecosystems of southern China, we collected an interesting fungus on dead branches of an unidentified broadleaf tree in Meiling National Forest

Park of Jiangxi Province, China. Close examination showed remarkable differences from all previously described hyphomycetes (Seifert & al. 2011), and its morphological identity was confirmed by molecular phylogenetic analyses. It is proposed here as a new genus, *Neoveronaea*.

Materials & methods

Isolates and morphological analyses

Samples of dead branches were collected from humid environments and a riverbank in Meiling National Forest Park (Jiangxi Province, China) and returned to the laboratory in Ziploc™ bags. Samples were processed and examined following the methods described in Ma & al. (2011). Fungi were mounted in a drop of lactic acid on microscope slides and examined and photographed with an Olympus BX 53 microscope with a 100× oil immersion objective. Adobe Photoshop 7.0 was used to assemble photographs into images. Single-spore isolates were cultured on potato dextrose agar (PDA) following Goh (1999). Colony colors were coded according to Rayner (1970). All fungal strains were stored in 10% sterilized glycerin at 4°C for further studies. The studied specimens were deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, China (HJAUP), and cultures were deposited in the Jiangxi Agricultural University Culture Collection (JAUCC).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fungal mycelia grown on PDA using the Solarbio Fungi Genomic DNA Extraction Kit following the manufacturer's protocol. The DNA was amplified by polymerase chain reaction (PCR), using the primer pairs ITS5/ITS4 (White & al. 1990) for the internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS), and 28S1-F/28S3-R (Ma & al. 2015) for the partial large subunit (LSU) nrRNA gene. PCR reaction volumes were 25 µl containing 1 µl DNA template, 1 µl of each forward and reverse primer, 12.5 µl 2× Power Taq PCR MasterMix, and 9.5 µl ddH_2O. PCR thermal cycling conditions were initialized at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 50 s, elongation at 72°C for 1 min, a final extension at 72°C for 10 min, and then stored at 4°C. The PCR products were checked on 1% agarose gel electrophoresis stained with ethidium bromide. Purification and DNA sequencing were carried out at Beijing Tsingke Biotechnology Co., Ltd. China.

Sequence alignment and phylogenetic analyses

Phylogenetic analyses were performed on concatenated ITS and LSU sequence data. Sequences generated from this study were analyzed with other similar sequences obtained from GenBank (Table 1). The sequences (Table 1) were initially aligned using MAFFTv.7 (Katoh & Standley 2013) on the online server (http://maffTh.cbrc.jp/alignment/server/) and optimized manually when

Table 1. Isolates and sequences used in the phylogenetic analyses. New sequences are in bold.

Species	Voucher	Accession no.		Reference
		ITS	LSU	
Cladophialophora carrionii	CBS 160.54	EU137266	FJ358234	de Hoog & al. 2007, Gueidan & al. 2008
C. parmeliae	CBS 129337	JQ342180	JQ342182	Diederich & al. 2013
	CBS 132232	JQ342180	JX081671	Diederich & al. 2013
Cyphellophora oxyspora	CBS 698.73	KC455249	KC455262	Réblová & al. 2013
C. sessilis	CBS 243.85	EU514700	EU514700	Réblová & al. 2013
Exophiala jeanselmei	CBS 507.90	—	FJ358242	Gueidan & al. 2008
E. nigra	dH 12,296	NR111131	FJ358244	Gueidan & al. 2008
E. pisciphila	AFTOL-ID 669	—	DQ823101	Gueidan & al. 2008
E. salmonis	AFTOL-ID 671	NR121270	EF413609	Geiser & al. 2006
E. xenobiotica	CBS 115831	AY857539	FJ358246	Gueidan & al. 2008
Fonsecaea monophora	CBS 102243	EU938579	FJ358247	Gueidan & al. 2008, Najafzadeh & al. 2009
Minimelanolocus aquaticus	MFLUCC15-0414	KR215607	KR215612	Liu & al. 2015
M. curvatus	MFLUCC15-0259	KR215605	KR215609	Liu & al. 2015
M. melanicus	MFLUCC15-0415	KR215608	KR215613	Liu & al. 2015
M. submersus	KUMCC15-0206	KX789212	KX789215	Liu & al. 2015
M. thailandensis	MFLUCC15-0971	MG922573	MG922577	Dong & al. 2018
Neoveronaea sinensis	**JAUCC M0840-1**	**OM832969**	**OM832968**	**This study**
	JAUCC M0840-2	**OM833054**	**OM832967**	**This study**
Phialophora macrospora	MUCL 9760	AF050281	AF050281	Untereiner & Naveau 1999
Ramichloridium anceps	AFTOL-ID 659	—	DQ823102	James & al. 2006
Thysanorea papuana	CBS 212.96[1]	NR111276	—	Arzanlou & al. 2007
	CBS 212.96[2]	EU041814	EU041871	Arzanlou & al. 2007
Veronaea botryosa	CBS 254.57	EU041816	EU041873	Arzanlou & al. 2007
V. compacta	CBS 268.75	EU041819	EU041876	Arzanlou & al. 2007
V. japonica	CBS 776.83	EU041818	EU041875	Arzanlou & al. 2007

[1]Ex-holotype culture.

[2]Ex-type culture.

needed. Phylosuite software v1.2.1 (Zhang & al. 2020) was used to construct the phylogenetic tree based on ITS + LSU sequence data. The concatenated aligned dataset was analyzed separately using Maximum likelihood (ML) and Bayesian inference (BI) methods. Maximum likelihood phylogenies were inferred by using IQ-TREE (Anisimova & al. 2011; Nguyen & al. 2015) under the Edge-linked partition model for 1000 standard bootstraps. For both analyses, the optimal ML

tree search was conducted with 1000 separate runs using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing the likelihood scores using the TIM2+F+I+G4 substitution model. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist & al. 2012) under the partition model (2 parallel runs, 2,000,000 generations), in which the initial 25% of sampled data were discarded as burn-in. The best-fit model was GTR+I+G for ITS and LSU. These trees were viewed in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree) and the trees were created in Adobe Illustrator CS v. 5. Newly generated sequences were deposited in GenBank. In this study, the recommendations of Jeewon & Hyde (2016) were followed to determine a new genus.

Phylogenetic results

The ITS and LSU phylogenetic analyses indicated the relationships between the new genus *Neoveronaea* and its morphologically similar genera within the *Herpotrichiellaceae* (Fig. 1). The concatenated aligned dataset comprises

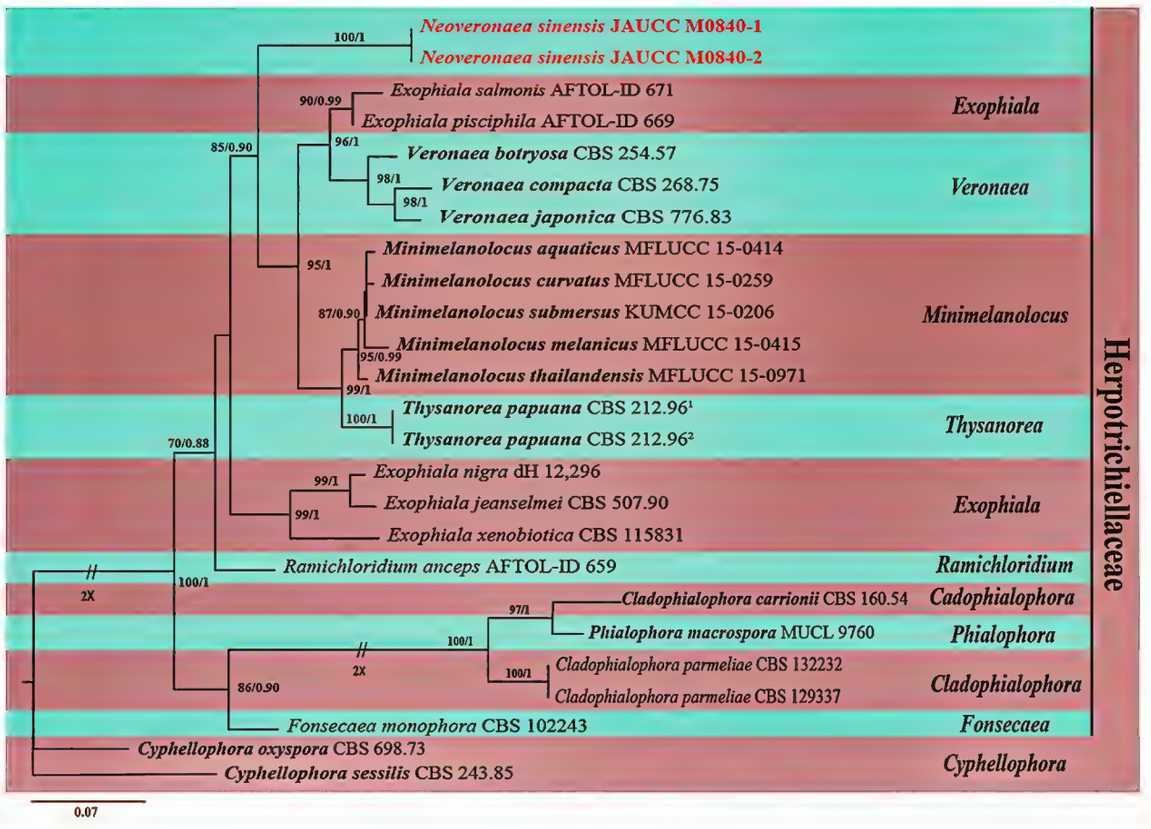

Fig. 1 Phylogenetic tree inferred from maximum likelihood and Bayesian inference analysis based on a concatenated alignment of ITS and LSU sequences. Significant ML/BI bootstrap support values are shown at the nodes. Ex-type isolates are in bold, and newly generated sequences are indicated in red. The tree is rooted to *Cyphellophora sessilis* (CBS 243.85) and *C. oxyspora* (CBS 698.73).

25 isolates representing 22 species, including *Cyphellophora oxyspora* (CBS 698.73) and *C. sessilis* (CBS 243.85) as the outgroup. Maximum likelihood and Bayesian Inference analyses of the combined dataset resulted in phylogenetic reconstructions with largely similar topologies, and the best scoring RAxML tree is shown in Fig. 1. Our two strains clustered with species of *Exophiala*, *Veronaea*, *Minimelanolocus*, and *Thysanorea*, forming a distinct clade with moderately strong bootstrap support (ML = 85%; BI = 0.90) within *Herpotrichiellaceae,* supporting our strains as represented a new genus in that family.

Taxonomy

Neoveronaea L. Qiu, K. Zhang, R.F. Castañeda & Jian Ma, **gen. nov.**

IF 559597

Differs from *Veronaea* by its conidiogenous cells with inconspicuous, slightly prominent, minutely denticles.

Type species: *Neoveronaea sinensis* L. Qiu & al.

Etymology: *Neoveronaea* = "*neo-*" + "*veronaea*" (Lat.), in reference to its similarity to the genus *Veronaea*.

Colonies on substrate natural effuse, pale brown to dark brown; mycelium partly immersed in the substrate, composed of branched, septate, smooth, pale brown to brown hyphae. Conidiophores macronematous, mononematous, erect, straight or slightly flexuous, cylindrical, unbranched, septate, smooth, brown to dark brown. Conidiogenous cells polyblastic, integrated, terminal and intercalary, sympodially extending, with tiny denticles, pale brown. Conidial secession schizolytic. Conidia solitary, acropleurogenous, obovoid to ellipsoidal, euseptate, smooth, pale brown.

Neoveronaea sinensis L. Qiu, K. Zhang, R.F. Castañeda & Jian Ma, **sp. nov.** Fig. 2

IF 559598

Differs from *Veronaea* spp. by its conidiogenous cells with inconspicuous, slightly prominent, tiny denticles, and conidia obovoid to ellipsoidal, 1–3-euseptate.

Type: China, Jiangxi Province: Nanchang, Meiling National Forest Park, 28.7760°N 115.7326°E, on dead branches of an unidentified broadleaf tree, 10 July 2020, L. Qiu (**Holotype**, HJAUP M0840; ex-type living cultures [metabolically inactive by lyophilization] JAUCC M0840-1, M0840-2; GenBank OM832967, OM832968, OM832969, OM833054).

Etymology: refers to the country in which the fungus was collected.

Colonies effuse, scattered, pale brown to dark brown. Mycelium superficial, and immersed in the substrate, composed of branched, septate, pale brown to

brown, smooth hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight or slightly flexuous, cylindrical, septate, brown to dark brown, paler towards the apex, smooth, thick-walled, 85–120 × 2.5–5 µm. Conidiogenous cells polyblastic, integrated, terminal and intercalary, indeterminate, sympodially extending, cylindrical, with inconspicuous tiny denticles, pale brown, smooth. Conidial secession schizolytic. Conidia solitary, acropleurogenous, dry, obovoid to ellipsoidal, base obconical-truncate, apex rounded, 1–3-euseptate, pale brown, smooth, dry, 7.5–14.5 × 2–5.5 µm.

Culture characteristics: Colonies on PDA media, reaching 30 mm diameter in 20 days at 25°C, buff-yellow, reverse dark olivaceous, velvety, dry, fairly dense, margin entire to sub-undulate.

Discussion

Neoveronaea is unique in its macronematous unbranched conidiophores and solitary, acropleurogenous, euseptate, obovoid to ellipsoidal, pale brown, smooth conidia that secede schizolytically from polyblastic, sympodially extending, terminal and intercalary, integrated conidiogenous cells with inconspicuous, slightly prominent, tiny denticles. It superficially resembles *Veronaea* Cif. & Montemart., but *Veronaea* differs by its cicatrized conidiogenous cells with flattened scars that are faintly pigmented and not thickened (Ellis 1971). *Neoveronaea* is also similar to the genera *Minimelanolocus* R.F. Castañeda & Heredia, *Dactylaria* Sacc., *Pleurophragmium* Costantin, *Rhodoveronaea* Arzanlou & al., and *Veronaeopsis* Arzanlou & Crous in conidial ontogeny, but *Minimelanolocus* differs in its conidiogenous cells with inconspicuous or slightly prominent, narrow, opaque, refractive to somewhat obscure conidiogenous loci (Castañeda-Ruíz & al. 2001). *Dactylaria, Pleurophragmium*, *Rhodoveronaea*, and *Veronaeopsis* differ by their terminal conidiogenous cells with prominent denticles (Ellis 1971, Arzanlou & al. 2007, Seifert & al. 2011). Both morphological and molecular data support our monotypic *Neoveronaea* as a new genus.

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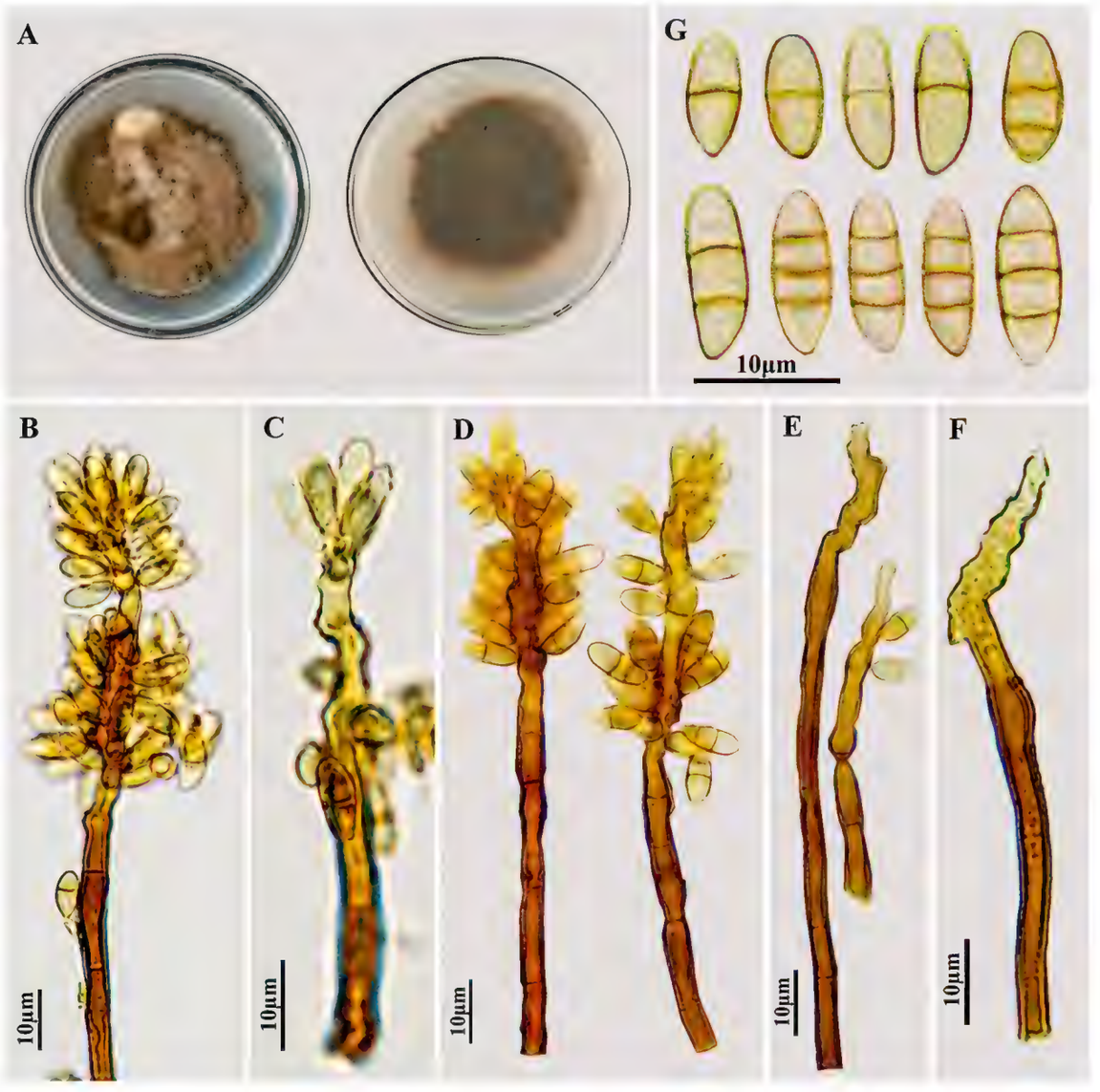

Fig. 2 *Neoveronaea sinensis* (holotype, HJAUP M0840). A. Colony on PDA (surface and reverse); B–D. Conidiophores without the basal part, conidiogenous cells, and conidia; E. Conidiogenous cells and conidia; F. Conidiogenous cell; G. Conidia.

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